TGF-β induced epithelial-mesenchymal transition plays a pivotal role in appendage-specific regenerative response of the lizard, *Hemidactylus flaviviridis*

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ABSTRACT

Wound healing is repairing injury via amalgamation of various cellular and molecular events. There are two predominant types of wound healing amongst vertebrates, fibrotic wound healing, causing a permanent scar at the culmination of healing and scar-free healing (super healing) that preludes regeneration of lost tissues. One terrestrial vertebrate (amniote) which displays both these modes of wound healing is the lizard, *Hemidactylus flaviviridis* (northern house gecko). The tissue architecture of the lizard tail has pre-determined fracture planes, which allow the tail to shed by autotomy, followed by scar-free healing leading to tail regeneration. In contrast, the severed limb follows scarred healing owing to collagen deposition and hence does not regenerate. Multiple processes such as angiogenesis, cell proliferation, and EMT (epithelial-mesenchymal transition) contribute to the complex yet dynamic process of wound healing. Yet, EMT's contribution towards pro-regenerative wound healing is under-researched. Furthermore, various molecules of the FGF family, Wnt family, and TGF-β family are known to induce EMT during cancer and wound healing. Nonetheless, the mechanism behind the site-specific occurrence of TGF-β indued EMT during differential wound healing of the lizard's appendages is yet to be elucidated. Thus, we analyzed the influence of TGF-β induced EMT on the wound-healing contrast of the lizard's appendages through a multi-tiered approach involving morphological observation, histological analysis, transcript level analysis, protein level analysis, and FACS analysis. Our findings demonstrated blastema formation in the tail, whereas, collagen deposition and scarring occurred in limb. Tissue architecture corroborated EMT in tail (epithelial cells close to mesenchyme were less columnar, loosely packed with little intercellular matrix). However, limb's epithelial cells retained their native shape (tightly packed and remained as a continuous sheet). Additionally, collagen deposition intersected wound epithelium and mesenchyme in limb, hindering EMT and promoting scarring. Furthermore, the transcript and protein levels showed upregulation of N-cadherin, Vimentin, PCNA, Sox2A and Nanog, (mesenchymal, cell migration, proliferation and pluripotency markers respectively) in regenerating tail, substantiating EMT. While scarring

limb depicted upregulation of epithelial markers E-cadherin and EpCAM. FACS results also showed elevated levels of Nanog in the tail's epithelial tissue, providing evidence for the formation of epithelial stem cells derived regenerative blastema. Along with the markers of EMT and stem cells, the TGF-β isoforms and downstream signalling molecules were also assayed. A stark difference in the temporal expression pattern of TGF-β1, TGF-β2, TGF-β3, TGF-βR1, TGF-βR2, PI3K, Akt, Smad2, Smad4, Smad7, Snail1, Twist1, MMP2. MMP9 and TIMP3 were noted between the tail and limb. Also, the presence of MMPs and TIMP3 was confirmed by the results of gelatin zymography and reverse zymography. Thus, the study of the temporal expression pattern of TGFβ isoforms and downstream effector molecules aided in unravelling their contribution to EMT which provided inputs to gain a better understanding of the mechanistic details of differential wound healing of the lizard's appendages (regenerating tail and scarring limb). Moreover, it can also plausibly be employed as a therapeutic target for the cure of wounds, in keloid treatment and also in regenerative medicine.

REFERENCES

1. Alibardi, L. (2010). Ultrastructural features of the process of wound healing after tail and limb amputation in lizard. Acta Zoologica, 91(3), 306-318.
2. Beanes, S. R., Dang, C., Soo, C., & Ting, K. (2003). Skin repair and scar formation: the central role of TGF-[beta]. Expert reviews in molecular medicine, 5(8), 1.
3. Foroutan, M., Cursons, J., Hediyeh-Zadeh, S., Thompson, E. W., & Davis, M. J. (2017). A transcriptional program for detecting TGFβ-induced EMT in Cancer. Molecular Cancer Research, 15(5), 619-631.
4. Houghton, P. E., Keefer, K. A., & Krummel, T. M. (1995). The role of transforming growth factor-β in the conversion from "scarless" healing to healing with scar formation. Wound Repair and Regeneration, 3(2), 229-236.
5. McCusker, C., Bryant, S. V., & Gardiner, D. M. (2015). The axolotl limb blastema: cellular and molecular mechanisms driving blastema formation and limb regeneration in tetrapods. Regeneration, 2(2), 54-71.
6. Ocaña, O. H., & Nieto, M. A. (2010). Epithelial plasticity, stemness and pluripotency. Cell research, 20(10), 1086-1088.
7. Sader, F., Denis, J. F., Laref, H., & Roy, S. (2019). Epithelial to mesenchymal transition is mediated by both TGF-β canonical and non-canonical signaling during axolotl limb regeneration. Scientific reports, 9(1), 1-13.

8. Subramaniam, N., Petrik, J. J., & Vickaryous, M. K. (2018). VEGF, FGF-2 and TGFb expression in the normal and regenerating epidermis of geckos: implications for epidermal homeostasis and wound healing in reptiles.
9. Thiery, J. P., Acloque, H., Huang, R. Y., & Nieto, M. A. (2009). Epithelial-mesenchymal transitions in development and disease. cell, 139(5), 871-890.
10. Wendt, M. K., Allington, T. M., & Schiemann, W. P. (2009). Mechanisms of the epithelial–mesenchymal transition by TGF-β. Future Oncology, 5(8), 1145-1168.
11. Xu, J., Lamouille, S., & Derynck, R. (2009). TGF-β-induced epithelial to mesenchymal transition. Cell research, 19(2), 156-172.
12. Xue, M., & Jackson, C. J. (2015). Extracellular matrix reorganization during wound healing and its impact on abnormal scarring. Advances in wound care, 4(3), 119-136.